NanoEntek

NESPI-FRCOA-002EN(V.0.0)

CE IVD

FREND™ COVID-19 total Ab

Qualitative assay for COVID-19 total antibodies

REF FRCOA 020

IVD For *in vitro* diagnostic use only

℞ For prescription use only. For Emergency Use Authorization only.

Intended Use

The FREND™ COVID-19 total Ab is a fluorescence immunoassay (FIA) using the FREND™ System intended for the qualitative detection of total antibody to SARS-CoV-2 in human plasma. The FREND™ COVID-19 total Ab is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C §263a, to perform moderate or high complexity tests and as applicable, point-of-care (POC) testing.

Results are for the detection of SARS-CoV-2 antibodies. Total antibody to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time antibodies are present post-infection is not well characterized. Individuals may have detectable virus present for several weeks following seroconversion.

Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

The sensitivity of FREND™ COVID-19 total Ab early after infection is unknown. Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.

False positive results for FREND™ COVID-19 total Ab may occur due to cross-reactivity from pre-existing antibodies or other possible causes. Due to the risk of false positive results, confirmation of positive results should be considered using second, different total antibody assay.

The FREND™ COVID-19 total Ab is only for use under the Food and Drug Administration's Emergency Use Authorization.

Summary and explanation of test

COVID-19 is a respiratory disease caused by a novel coronavirus called SARS-CoV-2. The symptoms for patients have included mild to severe respiratory illness with fever, cough, and difficulty breathing.[1-2] The test result shows the presence of SARS-CoV-2 antibodies, are generally detectable in the blood several days after symptom onset.[3-4] The antibody test provides information specific to an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. Presence of antibody may not be an indicator of immunity. Positive results should be confirmed by a second serology test. Final recommendations and patient management should be determined by healthcare professional based on clinical symptoms and other diagnostic tests.

Principle of the assay

The FREND™ cartridge utilizes micro-fluidics lateral flow technology where the analyte of interest in the sample forms immune complexes while moving through the fluidics pathway in the cartridge.

A specimen is added to sample dilution tube and mixed. A well-mixed sample of 35 µL is transferred to the sample inlet of a single use FREND™ COVID-19 total Ab cartridge. The cartridge is then placed into the FREND™ System, which is programmed to begin analysis once the sample has reacted with the reagents. The reaction and analysis time is approximately 3-4 minutes. The anti-coronavirus total antibody qualitative measurement is based on the ratio of fluorescence detected by the FREND™ System at the FREND™ Test and Reference zones. The magnitude of the fluorescent ratio is proportional to the presence and absence of total antibody in the sample.

The FREND™ System is a bench-top fluorescence reader containing a touchscreen user interface. The System has a slot that accepts the FREND™ COVID-19 total Ab test cartridge (which contains the reagents and sample) and is programmed to analyze the test when the sample has fully reacted with the on-board cartridge reagents. Results of the test are displayed on the screen and can be printed on an optional printer. The FREND™ System has been previously cleared for use with other FREND™ cartridges through the 510k process. Please see k162378 (FREND™ PSA Plus reagent cartridge), k162754 (FREND™ Vitamin D test system), k153577 (FREND™ Testosterone test system), k152422 (FREND™ Free T4 test system) and k131928 (FREND™ TSH test system).

Material provided

Q'ty	Contents	Catalogue number
20	Cartridges	FRCOA 020
20	Dilution tubes	
40	Disposable pipette tips	
01	Code chip	
01	Package insert	

Components required but not included with the test

The following materials are not provided with the reagent but are required to perform COVID-19 total antibody analysis using the FREND™ COVID-19 total Ab on the FREND™ System.
-FREND™ System including calibrated pipette, QC Cartridge and QC Code chip manufactured by NanoEntek.

Warning and Precautions

● The FREND™ COVID-19 total Ab cartridge are intended for *in vitro* diagnostic use only.
● The FREND™ COVID-19 total Ab cartridge are only to be used on the NanoEntek FREND™ System.
● The FREND™ COVID-19 total Ab cartridges and dilution tubes are disposable, single use devices. Do not reuse!
● Allow sealed cartridges to come to room temperature for 15-30 minutes prior to use.
● Cartridge and dilution tubes should not be frozen.
● The humidity in the laboratory must be 10-80% range when running tests.
● Avoid cross-contamination between samples by using a new pipette tip for each new specimen when transferring the sample to the dilution tube. Use another new pipette tip when transferring are diluted, sample to the cartridge.
● Avoid high humidity, direct sunlight or heat in the area used for cartridge storage.
● Inaccurate results are possible if the sample used is contaminated in any way.
● Using specimens containing clotted fibrin could result in erroneous results.
● Over or under loading the cartridge with sample may result in inaccurate results.
● Do not use the cartridges beyond the expiration date on the pouch.
● Do not use the cartridge if the pouch is damaged or the seal is broken.
● Perform testing as specified in the Package insert and User manual
● Keep the cartridge sealed in the pouch until ready for use.
● Use the cartridge immediately after opening the pouch.
● Handle specimens in accordance with the OSHA Standard on Bloodborne Pathogens.
● Human specimens are not used in the preparation of this product, however, since human specimens will be used for samples and other quality control products in the laboratory may be derived from human materials. Use Universal Precautions when handling all specimens and controls. Wear disposable gloves when handling the cartridges and the samples.
● Wash hands thoroughly and often after handling reagent cartridges or samples.
● Do not ingest the silica gel packet found in the cartridge pouch.
● Used cartridges and diluent tubes should be disposed in accordance with local or national regulations.

Storage and Stability

All unopened materials are stable until the expiration date on the label when stored at refrigerator temperature storage (2-8℃). Reagent stability has been demonstrated for twelve months from the date of manufacture.
The expiration date is clearly indicated on the product box and the cartridges.

Specimen collection and handling

The assay can be performed using EDTA plasma. No special patient preparation is necessary. Collect the appropriate venous blood sample in accordance with standard laboratory procedures. After collection, centrifuge the sample for 10 minutes at 3,000 rpm within 2 hours of collection and immediately separate the plasma from the packed cells.
It is recommended to use samples immediately. However, if testing is not done immediately, samples may be stored at 2-8℃ for up to 6 hours prior to testing. If testing is not performed within 6 hours, store at -20℃ or below. Samples can be stored frozen for up to 30 days prior to testing.

Repeated freeze-thaw cycles should be avoided. Turbid samples containing particulate matter such

as fibrin clots or visible strands should be-centrifuged before being tested. Prior to assay, slowly bring frozen samples to room temperature and mix gently but thoroughly before testing.
For optimal results, avoid grossly hemolytic, lipemic, or turbid specimens. Specimens should be free of aggregated fibrin, red blood cells, or other particulate matter. When pipetting into the cartridge sample inlet, ensure that bubbles in the sample are avoided. Bubbles may restrict flow and result in an incomplete or erroneous test result.

Procedure

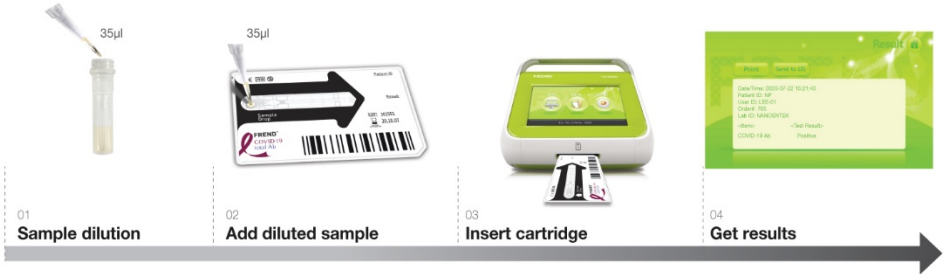
● Reagent preparation
There is no reagent preparation required to run the FREND™ COVID-19 total Ab cartridge on the FREND™ System. However, the cartridge needed for a particular run should be removed from the refrigerator and allowed to reach room temperature for 15-30 minutes before they are used.

● Code chip installation
A lot-specific Code chip is supplied with each kit of FREND™ COVID-19 total Ab. When using a new lot of reagent, the Code chip of the same lot must be installed in the FREND™ System. Please refer to the FREND™ System User manual for more detailed instructions relative to the Code chip installation. Abbreviated instructions are as follows:

(1) Insert the FREND™ System electrical cord into an appropriate outlet.
(2) Insert the Code chip into the Code chip slot at the rear of the system following the arrows.
(3) Press the 'Setup' button on the 'Main' screen.
(4) Press the 'Code chip' button on the 'Setup' screen.
(5) The information embedded on the FREND™ COVID-19 total Ab Code chip is automatically saved on the FREND™ System.
(6) When the Code chip installation is completed, press the 'OK' button to go to the 'Setup' screen.
(7) Press the 'Item' button on the 'Setup' screen.
(8) Check the FREND™ COVID-19 total Ab cartridge lot number and the installation date of the Code chip.
(9) Press the 'Home' button to go to the 'Main' screen to begin running external quality control and patient samples.

● Specimen processing
Allow the tubes and the sealed pouches containing the FREND™ COVID-19 total Ab cartridges and dilution tubes to come to room temperature for 15-30 minutes prior to use.
If using refrigerated patient samples, remove those from the refrigerator and allow to them to come to room temperature prior to testing. If frozen samples will be utilized, be sure these are removed from the freezer, thawed naturally and then mixed gently but thoroughly prior to testing. Testing should not begin on frozen samples until they have reached room temperature.

● Assay procedure



(1) Have the FREND™ COVID-19 total Ab cartridge and samples ready for testing.
(2) Record the Sample ID on the cartridge in the designated area.

(3) Using the micropipette, transfer 35 µL of the sample to a sample dilution tube and mix by inverting the sample gently for 3-5 times. Using the mixed sample and a new pipette tip, transfer 35 µL into the sample inlet.

(4) Press the 'Test' button on the 'Main' screen of the FREND™ System.

(5) The system moves to the Patient ID screen automatically.

(6) Type the Patient ID and press the 'Enter' button to begin the test.

(7) Insert the cartridge into the cartridge slot using the cartridge arrows as a guide.

Caution: Please check the direction of the cartridge before insertion and assure the insertion is complete.

Caution: Please insert the cartridge into the FREND™ System after loading the sample into the cartridge.

(8) When the reaction in the cartridges is complete, the FREND™ System will automatically begin the reading.

(9) When the reading has been completed, the cartridge will automatically be expelled and the results displayed.

Caution: Do not remove power from the FREND™ System while a cartridge is in the reading chamber. This may cause a system error.

(10) If the FREND™ System is connected to the optional printer, press the 'Print' button and the results will be output on the printer paper.

(11) For more detailed instructions, please refer to the 'FREND™ System User manual'.

Control materials

● FREND™ System check

It is recommended for operators to use the QC Cartridge daily for the maintenance of the FREND™ System. Install QC Code chip in the FREND™ System before using the QC Cartridge. The QC Cartridge confirms the proper function of the FREND™ System including:

-Step 1 Laser power

-Step 2 Laser alignment

-Step 3 Calculate ratio

Please use the QC Cartridge and QC Code chip provided with the FREND™ System.

● Internal control

FREND™ COVID-19 total Ab cartridge contains built in control features. Fluorescence signal in the Reference zone of each cartridge shows: (1) that enough sample volume is added, (2) that proper flow is obtained, and (3) that the antibody is reactive. If this Reference zone signal is missing or lower than the threshold, FREND™ System considers it as an incorrect or failed test, and produces an error message instead of a test result. In addition, with each cartridge run, the system monitors, in part, for (1) flow of sample, (2) speed of sample flow, (3) shelf-life of cartridge components, (4) function of internal barcode scanner, and (5) function of scanner's mechanical components.

● Positive and negative controls

Commercially available controls may be used. Good laboratory practice suggests that positive and negative controls are run routinely to ensure that test reagents are working and that the test is correctly performed. External positive and negative controls should be used in accordance with local, state, federal accrediting organizations, or your laboratory's standard quality control procedures, as applicable.

Interpretation of results

Positive results
A positive result is an indication of antibodies raised to the SARS-CoV-2 virus.

Negative results
Negative test indicate that total antibody to COVID-19 were not detected.

FREND System results display
FREND™ System qualitatively detection of total antibody to the SARS-CoV-2 virus. Report options are indicted below: Note the following precautions:
-Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus. Follow-up testing with a molecular assay should be considered to rule out infection in these individuals.
-Results from antibody testing are not an indication of SARS-CoV-2 infection. Presence of SARS-CoV-2 antibodies are an indication of potential immunity to the virus.
-Positive results may indicate past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E. Positive results should be confirmed using a second serology test.
-Not for the screening of donated blood.

Please read the section 'Result interpretation' and 'Limitation of procedure' carefully before you use the FREND™ COVID-19 total Ab and the FREND™ System.

Displayed results	Description
	COVID-19 Ab "Positive"
	COVID-19 Ab "Negative"

Limitation of the procedure

● This test is for clinical laboratory use only. Not for home use.
● This test cannot rule out diseases caused by other bacterial or viral pathogens.
● This test must be read using only the FREND™ System.
● Immunocompromised patients who have COVID-19 may have a delayed antibody response and produce levels of antibody which may not be detected as positive by the assay.
● The assay procedure and results interpretation must be followed closely when testing for the presence of SARS-CoV-2 virus specific antibodies. For optimal test performance, proper sample collection is critical. Failure to follow the procedure may give inaccurate results.
● If symptoms persist and the result of the COVID-19 total Ab test is negative or non-reactive, it is recommended to re-sample the patient a few days later or test with an alternative test device.
● The results obtained with this test should only be interpreted in conjunction with clinical finding, and the results from other laboratory tests and evaluations.
● Heterophilic antibodies in serum specimens may cause interference in immunoassay. These antibodies may be present in blood samples from individuals regularly exposed to animals or who have been treated with animal serum products. Results that are inconsistent with clinical observations indicate the need for additional testing.
● This test should not be used for screening of donated blood.

Performance evaluation

Analytic sensitivity and specificity

a. Reactivity/ inclusivity

Although mutations in the SARS-CoV-2 genome have been identified as the virus has spread, no serologically unique strains have been described relative to the originally isolated virus (this research is exceptionally limited at present.)

b. Cross-reactivity

Testing was performed on samples collected from the United Stated of America. Blood samples (EDTA plasma) were purchased from Gulf Coast Regional Blood Center 1400 La Concha Lane Houston, Texas 77054 on August 20, 2019 before the outbreak of COVID-19.

Method		Prior to COVID-19
FREND™ COVID-19 total Ab	Positive	0
	Negative	120
Total		120 (100%)

Clinical agreement study

Clinical samples were collected at Kangwon National University Hospital, Department of diagnostic examination, 156 Baeknyeong-ro, Chuncheon-si, Gangwon 24289, Korea from April 7th to April 10th 2020. Patients were confirmed for SARS-CoV-2 by rtPCR with the Allplex™ 2019-nCoV Assay (Seegene, Inc.). The results were as follows.

Method		RT-PCR confirmed	
		Positive	Negative
FREND™ COVID-19 total Ab	Positive	72	1
	Negative	3	15
Total		75	16

-Positive percent agreement: 96.0% (72/75), 95% CI (88.89-98.63)
-Negative percent agreement: 93.8% (15/16), 95% CI (71.67-98.89)

An additional 56 clinical samples (44 SARS-CoV-2 PCR confirmed and 12 negative), purchased from a commercial vendor in the United States were tested at an independent clinical laboratory with the FREND COVID-19 total Ab. The results are presented in the table below.

Method		RT-PCR confirmed	Prior to COVID-19
		Positive	Negative
FREND™ COVID-19 total Ab	Positive	44	2
	Negative	0	10
Total		44	12

*Sample was negative upon re-testing
-Positive percent agreement: 100.0% (44/44), 95%CI (91.97-100.00)
-Negative percent agreement: 83.3% (10/12), 95% CI (55.19-95.30)

An additional 200 clinical samples (100 SARS-CoV-2 PCR confirmed and 100 negative), purchased from MRN Diagnostic, LLC in the United States were tested at the NanoEntek laboratory with the FREND™ COVID-19 total Ab. The results are presented in the table below.

Method		RT-PCR confirmed	Prior to COVID-19
		Positive	Negative
FREND™ COVID-19 total Ab	Positive	100	0
	Negative	0	100
Total		100	100

-Positive percent agreement: 100.0% (100/100), 95%CI (91.97-100.00)
-Negative percent agreement: 100.0% (100/100), 95%CI (91.97-100.00)

The combined data for all US and Korean testing are presented in the table below:

Method		RT-PCR confirmed/prior to COVID-19	
		Positive	Negative
FREND™ COVID-19 total Ab	Positive	216	3
	Negative	3	125
Total		219	128

-Positive percent agreement: 98.6% (216/219), 95%CI (92.85-99.14)
-Negative percent agreement: 97.7% (125/128), 95% CI (94.21-99.31)

Day between		219 positive confirmed by RT-PCR				
		0~7 days	8~14 days	15~21 days	>21 days	UNK
FREND™ COVID-19 total Ab	Positive	34	25	13	135	9
	Negative	3	0	0	0	0
Total		37	25	13	135	9
PPA		(34/37) 91.9%	(25/25) 100.0%	(13/13) 100.0%	(135/135) 100.0%	(9/9) 100.0%

Interference

The interference evaluation test of FREND™ COVID-19 total Ab was conducted according to CLSI Guidelines EP7-A2 using one lot.
No interference in the testing of the FREND™ COVID-19 total Ab with 4 interfering substances was observed.

Endogenous substances	Concentration tested
Bilirubin	20 mg/dL
Hemoglobin	500 mg/dL
Triglycerides	3 g/dL
Total protein	12 g/dL

References

1. Coronavirus Disease 2019 (COVID-19) Frequently Asked Questions, FDA
2. Q&A on coronaviruses (COVID-19), WHO
3. SARS-CoV-2: virus dynamics and host response, Yu Chen, Lanjuan Li, Lancet, March 23, 2020, https://doi.org/10.1016/S1473-3099(20)30235-8
4. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study, Kelvin Kai-Wang To, *et al.*, The Lancet Infectious Diseases, March 23, 2020, https://doi.org/10.1016/S1473-3099(20)30196-1

Glossary of symbols

	Caution, warning, Consult accompanying documents		*In vitro* diagnostic medical device
	Catalogue number /Reference number		Temperature limitation
	Lot number /Batch number		Contains sufficient for <n> tests
	Use by YYYY-MM-DD or YYYY-MM		Do not reuse
	Manufacturer		Do not use if package is damaged
	Authorized representative in the European Community		For prescription use
	CE marking		Irritant

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